PHARMACEUTICAL ANTIHERPETIC COMPOSITION AND A METHOD FOR PREPARING A DOSAGE FORM BASED THEREON

The present invention relates to the field of medicine, particularly to the provision of a new pharmaceutical antiherpetic composition and to preparing a dosage form based thereon

At present the following preparations against herpes virus are known: Acyclovir, Virazol, Foscarnet, Vidarabin, and others [1].

However, chemotherapeutic means are not always sufficiently effective, because they do not have a specific action with regard to herpes virus only, in principle are polyactive , and can be used in other virus diseases,.

Corresponding vaccines have specific activity with regard to herpes virus. A number of antiherpetic vaccines, including those based on inactive virions, have been developed both in Russia and in other countries. For example, such vaccines are described in Russian Federation Patents No. 2014084, 1994, No. 2085582, 1997, No. 2085583, 1997, in US Patents No. 4816250, 1989, No. 5215745, 1993, No. 5602023, 1997.

Though the vaccines cited in these documents are specific and prepared on the basis of inactivated and attenuated strains of herpes virus, not all of them are fit for treating humans. For the combating a virus in a highly organized biological organism to be successful, it is necessary to take into account humoral and cellular factors, the condition of the immune system, the degree of affliction by the virus, the stage of the disease (latent infection, acerbation).

In due course, experiments with simplex herpes virus (SHV-1) which belongs to the subfamily Alphaherpesvirinae revealed its immunodepressive activity. Inhibition of T-cellular proliferation may be combined with as defect of humoral response to a heteroantigen. The complicated character of the interaction of the simplex herpes virus, serotype 2 (SHV-2) with the immune system of mice demonstrates the fact of enhancement of the sensitivity of the host to the infection with Coxsackie virus B, with the normal response to sheep red blood cells. Infection of alveolar or peritoneal macrophages leads to suppression of their ability to perform the function of antibody-dependent cytotoxicity effectors. At early stages of infection a temporary increase of the phagocytic activity was detected in alveolar macrophages, said increase being then succeeded by suppression of this function. The immunodepressive activity of the virus was investigated by simulating this effect in a

culture of peripheral mononuclear cells of human blood. After the infection and corresponding incubation natural killers (NK) were isolated and their cytotoxicity was determined. In the course of work with the described system it was established that the virus suppresses the cytotoxic function of NKs only when monocytes are present in the culture of peripheral mononuclear cells. Removal of the latter from the system cancels the development of the virus-induced suppression, after the introduction of monocytes the immunologic defect is formed again. It was established also that the development of one of the types of suppression conditioned by herpes viruses is realized via monocytes. There are data also about the direct action of herpes virus on regulatory T-cells, this leading to a disturbance of the synthesis and of the ability to interact with interleukin-2 [2].

The described mechanisms of action of the herpes virus on immunocompetent cells and on the immune status on the whole suggest that into antivirus preparations compounds shall be included, which specifically influence the immune system, as well as substances normalizing the cellular metabolism, when an organism has been afflicted by herpes simplex virus, especially in cases of sluggish, recurrent diseases, diseases that poorly answer to therapeutic treatment, have a tendency to chronization. It is expedient to include into the complex therapy immunocompetent substances, such as interferons and antioxidants, as recommended in our previous inventive developments (see, e.g., Russian Federation Patent No. 2142816, 1999), where one of dosage forms convenient for a patient (in the form of as suppository) is also shown. We regard said inventive development as the closest analog of the present invention.

In spite of considerable advantages offered by the above-named vaccine preparation, some important aspects of the condition of the patient's organism afflicted by herpes virus are not taken into account therein. In patients with chronic forms of the disease a disturbance of the cellular and humoral immunity is observed, an enhanced secretion of glucocorticoids and a sharp disturbance of the metabolism of the virus-affected cells and tissues take place.

It is a technical object of the present invention to provide a new, more effective and advanced comprehensive preparation which would not only selectively and strongly act on herpes simplex virus, but also provide an intensive immunomodulating effect on an organism as a whole, and also normalize the metabolism in virus-affected cells and tissues.

Said object is accomplished by that a virion vaccine antiherpetic preparation, wherein viruses of herpes simplex of serotypes 1 or 2 inactivated by formalin or γ-radiation and an acceptable physiological solution are comprised, further comprises polyoxydonium, and also valine, lysine, isoleucine, as well as a combination consisting of at

least 2 amino acids selected from the group consisting of phenylalanine, leucine, alanine, threonine, histidine, arginine, methionine. The pharmaceutical composition has the following ratio of the components:

antiherpetic preparation — 10^6 to 10^7 plaque-forming units/ml

polyoxydonium	0.03—0.06 g
valine	0.18—0.25 g
lysine	0.15—0.30 g
isoleucine	0.11—0.22 g
combination of 2 metabolic amino acids	0.12—0.27 g
physiologically acceptable solution	to 100 ml

The composition may further comprise a number of solid, soft, liquid adjuvants or a mixture thereof.

The composition may further comprise a combination of 2—3 water- and fat-soluble vitamins selected from the group consisting of thiamine, riboflavin, nicotinamide, pyridoxine, ascorbic acid, retinol, tocopherol, or their mixtures in a total amount in the formulation of the composition of from 0.05 to 3.5%.

Such preparation is suitable in treating herpes simplex of types 1 and 2. For this purpose a combination of amino acids and vitamins corresponding to each kind of herpes is selected, the dosage and schedule being selected depending on the condition of a patient.

The last-generation immunomodulator produced in the Russian Federation, polyoxydonium (PO), is a very effective immunocompetent formulation. PO is a copolymer of 1,4-etylenepiperazine N-oxide and (N-carboxyethyl)-1,4-ethylenepiperazinium bromide. It comprises a lyophilized porous mass with a yellowish hue, readily soluble in water, with a molecular mass of from 60,000 to 100,000. Besides the immunomodulating effect, PO has a pronounced detoxicating effect, an antioxidant effect and a membrane-stabilizing effect [3]

Lately we have undertaken a number of experiments with the use of PO simultaneously with using the antiherpetic vaccine administered jointly by way of parenteral introduction. These experiments gave positive results on model infections in laboratory animals (keratitis in rabbits, genital herpes in guinea pigs, meningoencephalitis in mice). The results of these experiments are presented in Table 1.

Table 1

Nos.	Herpes virus vaccines employed	Results of titrating test viruses in lg TCD_{50}/ml	Single-injection dose of Polyoxidonium in mg	Concentration of virus-neutralizing antibodies in neutralization indexes
1	Killed vaccine against herpes simplex virus Type 1	6.5	— 0.1 0.2	2.3 3.75** 3.3*
2	Killed vaccine against herpes simplex virus Type 1	5.5	— 0.1 0.2	2.0 3.0* 2.5
3	Killed vaccine against human cytomegalovirus	5.0	— 0.1 0.2	2.0 3.0** 2.5

Note:

The reliability of difference in the concentration of virus-neutralizing antibodies in vaccinated animals without use of Polyoxidonium and with the use thereof. $**P \leq 0.01$; $*P \leq 0.05$.

Our investigations have shown that the best effect for the test individuals with different forms of herpes is produced by the high-technology synthetic preparation Polyoxidonium (PO).

As is shown by the investigations, the choice of the immunomodulating preparation and of the schedule of its application to patients should be determined by an immunologist depending on the severity and kind of the main disease, the concomitant pathology, and also on the revealed (sometimes hidden) immunologic defect.

When cells of the monocyte/macrophage system are injured, polyoxydonium, licopid are used. In cases of most severe forms of injuries in the monocyte/macrophage system, preparations of granulocyte/macrophage colony-stimulating factors are used: molgramostim (leukomax), filgrastim (neupogen).

In the case of defects in the cell link of the immunity, one of the following preparations are used: polyoxydonium, T-activin, thymolptin, thymogen, thymolin. When the synthesis of antibodies is disturbed by B-lymphocytes, myelopid, polyoxydonium are used.

Among preparations with immunostimulating properties three main groups which are used in practical health care can be singled out: preparations of microbial origin (Pyrogenal, Prodigiosanum, Ribomunyl, Sodium nucleate and others), preparations of en-

dogenic origin: thymus preparations (T-activin, Thymolin, Thymoptin, Thymactid, Thymostimulin and others), preparations of marrow origin (Myelopid), cytokines (Molgramostin, Reaferonum and others), chemically pure, synthetic preparations: medicinal preparations with revealed immunostimulating properties (for example, diuciphonum and others), analogs of substances of endogenic origin (Licopid, Thymogen and others) synthetic preparations proper (polyoxydonium and others).

Our investigations have shown that the best effect for test subjects with different forms of herpes is the high-technology synthetic preparation polyoxydonium.

In vivo conditions PO produces a more sophisticated and multi-aspect effect on the immune system. Since the development of any immune response commences with the cells of the monocyte/macrophage system, and since cytokines produced by monocytes/macrophages have a pleiotropic effect, an enhancement of their functional activity under the influence of PO leads to activation of both cellular and humoral immunity. Thus, in particular, upon administering PO jointly with low doses of an antigen there takes place a 5- to 10-fold enhancement of the synthesis of antibodies to this antigen as compared with control. It is important to note that such enhancement can be observed in animals with a genetically determined weak reaction to the given antigen. Hence, PO has a capacity of driving all the factors of organism protection from foreign agents of antigen nature, and this driving propagates in a natural manner so as this occurs in the development of immune response in an organism. These observations have enabled us to stop our selection among a large number of present-day immunomodulators exactly at polyoxydonium for its successful use in the formulation of a complex pharmaceutical antiherpetic composition. We have succeeded to establish empirically that very effective components in the formulation of the composition are essential amino acids valine and lysine, and also certain amino acids which lead to the acceleration of epithelialization of virus-affected cutaneous tissue. Such a composition, especially with the addition of microelements, which enhances immune response to the administration of the preparation, makes it possible to use thereof also for a more effective combating of virus lesions caused by herpes of serotypes 1 and 2.

There exist the following mechanisms of action of microelements (ME) in an immune system:

There exist the following mechanisms of action of microelements (MEs) in the immune system:

1. *Action on Specific Receptors*

On receptors localized on cytoplasmic membrane: HLA-system, MNS-system (Ni, Cr, Hg).

Adhesins: selectins and integrins (Mn, Hg).

Receptors to transferrin (Al, Ga).

Receptors participating in EK-lysis (Zn).

Cytokine receptors (Zn).

T-cell receptor (Zn, Hg).

Receptors to calcium and magnesium ions (Zn, Mn, Be, Cd, Hg, and others.

Receptor to immunoglobulins (Zn).

To receptors localized on intracellular compartments:

Mitochondria (Fe, Zn), Cytoskeleton, LIM-proteins (Zn, Se, Li).

Intracellular receptors to calcium on mitochondria , endoplasmic reticulum (Cd, Zn).

2. *Influence on Enzyme Activity*

Many essential MEs are a component of the catalytic site of a number of enzymes. For example, Mn is an essential part of the superoxide dismutase (COD) of immunocytes, Se enters into the catalytic site of glutathione peroxidase (isoenzyme VI), Zn is the most important part of numerous finger proteins controlling the transcription level of other intracellular proteins. There exist also pathways of the action of MEs on the activity of enzymes, which pathways consist in competitive inhibition or allosteric activation of metalloenzymes. For example, Zn is a competitive inhibitor of Ca^{+2+}, Mg^{2+}-dependent endonuclease. This action of Zn has determined its leading role in the immune system as an antiapoptic factor.

3. Influence on Hormone Activity

- MEs as a component part of hormones.

Zn is a key component of thymosin, a hormone realizing the effects: of the thymus on the T-cell link of the immune system.

MEs and Storage of Hormones

Zn, Cr participate in the storage and stabilization of the molecule if insulin which produces multimodulating effect on all insulin-dependent cells of an organism, to which immunocytes belong too. Zinc provides intracellular storage and stabilization of neurohypophysis hormones.

Participation in Hormone Degradation and Elimination

It is known that angiotensin-converting enzyme is Zn-dependent.

Participation in the mechanism of hormone action.

4. Influence on Carrier Proteins

Albumins

Metallothionins which are synthesized in mononuclear cells of the reticulo-endothelial system of the organism.

Stress proteins, as universal proteins synthesized in cells in response to stress effects (thermal shock, hunger, UV irradiation, effect of heavy metals, chronic infection).

5. Physicochemical Action of MEs on Immunocyte Membranes

It has been found that, e.g., selenium can produce an antioxidant effect, acting as a cofactor of glutathione peroxidase which provides inactivation of free oxygen forms, the generation of which in the immune system ensures both destruction or elimination of a foreign agent (parasite, bacterium), and, in excess production of singlet $O_2^{\cdot}$, H_2O_2, $^{\cdot}OH$, causes damage of the membrane apparatus of the very immunocytes.. The origination of $O_2^{\cdot}$, H_2O_2, $^{\cdot}OH$ is associated with induction of Haber-Weiss and Fenton reactions under the influence of transition metals (Cu, Zn, Mn, Fe).

Thus, MEs are able through the agency of enzyme and non-enzyme mechanisms of lipid peroxidation (LPO), as well as through the activation of antioxidant mechanisms, to control the physicochemical properties of cell membranes, including the property of semi-permeability with regard to various biological substrates (antigens, infectious agents, etc.).

6. Effect on the presentation, intracellular processing and degradation of antigens (see Item 1).

7. Effect on the formation of immunologic memory, and also probably, in the long-term existence of memory cells anti-apoptic MEs (Zn, Se and others) are involved.

8. Effect on the production of immunoglobulins (Zn, Be).

9. Influence on the processes of chemotaxis, adhesion and phagocytosis [5, 6]

Numerous tests on laboratory animals have made it possible to select the most effective microelements for incorporation thereof into the formulation of the dosage form developed by us (suppository): 2—3 microelements selected from the group: zinc, chromium, selenium and nickel. The presence of said microelements in the formulation of the dosage form enhances the action of the immunomodulator selected by us by 25—30% over the control (without MEs).

Presented below is Table 2, which shows the level of immunologic indexes in experimental animals infected with herpes virus, before and after introducing Polyoxydonium into the formulation of the composition comprising amino acids, including isoleucine .

Table 2

Indexes	Before introducing Poly-oxydonium	After introducing Polyoxy-donium
Leucocytes (abs)	7.6 ± 0.2	7.5 ± 0.1 p > 0.05
Lymphocytes %	31.1 ± 1.9	31.8 ± 2.3 p > 0.05
CD 3 + %	58.5 ± 2.2	72.1 ± 1.3 p < 0.005
CD 4 + %	30.5 ± 1.1	41.4 ± 1.32 p < 0.005
CD 8 + %	18.0 ± 0.5	22.8 ± 0.7 p < 0.005
CD 16 %	8.7 ± 1.3	11.5 ± 1.2 p < 0.05
Ig A mg %	250 ± 6.5	265.2 ± 6.2 p > 0.05
Ig G mg %	1502.1 ± 31.3	1575.2 ±30.5 p > 0.005
Ig M mg %	175.7 ± 9.6	182.1 ± 7.8 p > 0.05
Neutrophil phagocytosis %	52.1 ± 2.3	67.3 ± 6.1 p < 0.005

From the Table the influence of PO on the immunologic indexes is seen, wherein the cell immunity and the level of immunocompetent proteins reliably increase. It should be noted that the incorporation into the composition of amino acids (valine and lysine, and also isoleucine, and further a combination consisting of at least 2 amino acids selected from the group: phenylalanine, leucine, alanine, threonine, histidine, arginine, methionine) in all the combinations proposed by us accelerated the re-epithelialization of affected tissues (from 15 to 36% over the prototype, see above),depending on the kind of virus). The incorporation of polyoxydonium into the composition jointly with amino acids not only activated the immunity, which made it possible to prolong considerably the period of remission, to accelerate the process of re-epithelialization and healing of skin , and in some cases to succeed practically in healing some chronic forms of the disease in individual animals (12% of all the individuals under test).

Another object of the invention is the provision of a method for preparing a suppository based on the above-named pharmaceutical composition with the aid of a conventional suppository-making technology, in which method into the mass of a suppository based on cocoa oil there are additionally introduced the active components of the above-described pharmaceutical composition by following a conventional technology, and 2—3

microelements selected from the group consisting of zinc, chromium, selenium and nickel. The set of microelements is introduced into the suppository formulation as soluble chelate forms in an amount of 0.01 to 0.08% for the total mass.

The results of testing produced suppositories on a group of 76 experimental animals showed that using the new preparation not only activated the immunity, but made it possible to prolong appreciably the period of remission, to accelerate the process of re-epithelialization and healing of skin and mucous tissues, and in some cases practically to succeed in cutting the disease in separate individuals (12% of all the test subjects with the chronic form of the disease).

In our opinion, no small part in this result was had by the incorporation into the suppository formulation of vitally important ingredients controlling the metabolic processes on all levels, namely, of: PO, valine and lysine, isoleucine stimulating the production of virus-blocking peptides (of cecropins type) by the intestinal cells, of vitamins of all groups, and also of MEs: zinc, chromium, selenium and nickel.

Main performance criteria. The time of attaining topical convalescence (complete re-epithelialization) reduced by 15—36%, the duration of remission increased on an average to 6—7 months, the absence of virus in the smear (PCR-diagnostics) in 98.7% of cases, activation of the antivirus immunity, practically complete curing in 12% of the follow-ups of the animals.

Among the test animals (herpetic keratitis in rabbits, genital herpes in guinea pigs) 52 individuals were affected with herpes simplex virus of serotypes 1 and 2 in acute form and 24 individuals were affected with chronic form of herpes simplex. The animals with the acute form were divided into three groups: in the 1st group treatment was carried out with the herpetic vaccine described in the prototype; in the 2nd group, with the new pharmaceutical composition; in the control group the animals received chemotherapy in the form of ointments and solutions containing the known antiherpetic preparations. The group of the animals with the chronic form received new suppositories with a complete set of amino acids, vitamins and microelements.

Results. In the 1st group complete re-epithelialization took place on an average on the 6th day of treatment, while in the 2nd group complete re-epithelialization was noted on an average on the 4th —5th day of treatment. In the control group complete topical convalescence was stated on an average on the 7th —10th day from the start of treatment. The virus in control smears was not detected in 95.2% of the 1st group, in 97.8% of the 2nd group, and in 92.5% of the control group. The remission duration to 4 months took place in 92% of cases in the first group and to 6—7 months in 85% of cases in the second group.

At the same time, in the control group the remission duration to 4 months took place only in 85%. A highly intensive immune response was registered in the 1st group in 92.1% of cases, while in the 2nd group this was in 98.6% with a clear-cut reliability ($p < 0.01$). Thus in the blood serum of the test animals of both groups (in the second group by 11—18% higher) after the treatment a tendency was noted to an increase in the percentage and absolute content of natural killer cells, T-lymphocytes of CD8+ phenotypes, and activation of virus-induced PI a (phagocytic index) mitogen-induced PI g.

The obtained pharmaceutical suspension taken in an amount of 20 ml is mixed with 0.01—0.03 ml of each of the solutions of salts of 2—3 MEs and cocoa oil to the total mass of 100 g, from the resulting mass suppositories, each suppository weighing 0.15—0.20 g, are prepared by a conventional method Such suppository contains ≤ 400 μg /g of protein, has a residual humidity ≤ 2.2% and physiological pH value 7.3 ± 0.2, is nontoxic and capable of inducing in rats the synthesis of virus-neutralizing antibodies, with the neutralization index equal to 3.0 lg TCD_{50}/ml in terms of HSV-1 and to 2.0 lg TCD_{50}/ml in terms of HSV-2, where TCD_{50} denotes the dose producing cytopathic effect in 50% of test tubes with a cell monolayer, infected with the virus.

Owing to the creation of the new highly immunogenic composition, the antigenicity and stability of the specific activity of the preparation are preserved, not only the antivirus properties are enhanced, but the organism resistance is increased and prolonged not only in acute but also in chronic infection.

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